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Distribution of R Plasmids Among the O-Antigen Types of *Escherichia coli* Isolated from Human and Animal Sources

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The O-antigen types of 600 independently isolated *Escherichia coli* strains from human feces have been determined, and the types have been related to the antibiotic resistance patterns of the strains. The relative abundance of each O-antigen type differed in the susceptible and resistant series of strains. The majority (86%) of the resistant strains carried R plasmids. Resistant *E. coli* (20.3%) were found associated with O-antigen types 8, 9 and 101, whereas the susceptible strains covered a wide range of O-antigen types. Examination of 174 resistant strains isolated from calf feces also showed a prevalence of O-antigen types 8, 9, 101 (24.1%), and it seems probable that strains expressing these three O-antigen types commonly carry R plasmids in the alimentary tracts of man and calves. The number of strains not typeable with the O sera available were similar in the human (12.5%) and the calf (11.5%) series. There are no grounds for distinguishing "human" from "calf" *E. coli* on the basis of their O-antigen reactions.

Antibiotic-resistant strains of *Escherichia coli* are now widespread in the environment. Not only do they complicate the treatment of some human diseases but they are also found in considerable numbers in the gut contents of normal, healthy people (5, 19). In addition, farm animals frequently carry considerable numbers of these organisms (13, 30), and they are also to be found in sewage (20), some rivers (14), and coastal waters (34, 35). Foodstuffs may be contaminated when conditions are favorable.

Undoubtedly the use of antibacterial agents by man for various purposes has had a major effect in building up this reservoir of resistant strains, and the use of antibiotics in farm animals has been singled out for particular comment and legislative control (28), since the high incidence of resistant strains in this particular part of the human environment has been identified as a possible source of bacteria potentially dangerous to human health (1). Certainly this reservoir has been the source of resistant *Salmonella* strains, and a similar origin for the resistant *E. coli* strains found in man has been suspected by many (27).

This view, however, is not universally held, and not everyone is convinced of the danger of the animal reservoir of resistant organisms for man (15). It has, for example, been claimed that "human" and "animal" *E. coli* are distinct (3), and also that the majority of strains found in man arise by self-contamination by the human population, either directly or indirectly through processed food (25, 31).

Although there have been several studies which compare the types of *E. coli* found in man and in farm animals (12), there have been no detailed studies on the serotypes of R plasmid-carrying *E. coli* and on the distribution of these various types in man and animals. We have therefore O antigen typed a relatively large number of independent *E. coli* strains from both sources to assess the extent to which the R plasmid-carrying flora overlap. Any overlap would, at least, indicate that the flora in farm animals cannot be neglected in studying the sources of resistant *E. coli* for man.

MATERIALS AND METHODS

Source of strains. A total of 600 strains isolated from human feces are included in this survey. Of these, 89 were isolated from hospitalized patients and the remainder from a large variety of sources including students and outpatients attending maternity clinics. In all, the survey involved 1,330 samples from which more than 15,000 individual strains were typed.

The strains of animal origin (264 in all) were obtained from calves kept under a variety of methods of husbandry in England. Samples were taken from 78 farms, and the strains were isolated from more than 500 samples; a total of 1,893 strains were O antigen typed.

Scoring of isolates. The isolation of a specific *E. coli* O-antigen type from a given fecal sample was

scored as one isolate. Any further isolates of the same O-antigen type made from the same fecal sample were discarded as duplicates. Strains with similar O-antigen type and resistance pattern, but isolated from different fecal samples from the same person or animal, or even from different people or animals who may have been in contact, were also discarded as possible reisolations of the same strains. This rigorous exclusion procedure reduced the 15,000 or so O-antigen typed human isolates in this study to the 600 actually included in the survey and the 1,893 calf isolates to 264.

Isolation of strains. The nutrient broth and agar, the MacConkey bile lactose broth and agar, and the peptone water used in these studies were all standard bacteriological growth media.

The human fecal strains were isolated as follows. Approximately 3 g of feces were collected on voiding in sterile plastic containers. If immediate processing was impossible samples were refrigerated at 4 C but always examined within 8 h of collection. About 1 g of material from the fecal samples was accurately weighed into a bottle, and enough physiological saline was added to give a 10% (wt/vol) suspension of material. This suspension was then homogenized, and 10-fold serial dilutions were prepared in sterile physiological saline down to a dilution of 10^{-7}. These dilutions were then used to inoculate duplicate series of MacConkey bile lactose agar plates with single drops (0.02 ml) by a modification of the method of Miles and Misra (23). Five plates were used in each series: one contained unsupplemented bile lactose agar, whereas the remaining four contained similar medium supplemented with either tetracycline (25 μg/ml); streptomycin (10 μg/ml); ampicillin (25 μg/ml); or chloramphenicol (25 μg/ml). After overnight incubation at 37 C, colony counts were made of those dilutions which showed between 20 and 50 colonies per drop. Single colonies obtained in this way were inoculated into nutrient broth and incubated for 2 h at 37 C before being used for further study. With each fecal sample, 10 lactose-fermenting colonies were picked from the unsupplemented bile lactose agar plates, and two colonies, where possible, were chosen from each of the antibiotic-containing plates. Representative, non-lactose fermenting colonies were also picked whenever they occurred.

Animal strains were isolated in a similar way to the human ones. Samples were taken from the rectum by hand either using a plastic sleeve which was everted over the samples and knotted or with a large alginate swab. The larger fecal samples were processed quantitively as with the human samples, whereas the rectal swabs were inoculated directly onto MacConkey bile lactose agar with and without added antibiotics.

Identification of strains. Biochemical tests for identification purposes were performed on all lactose-fermenting coliform colonies by inoculating 4 ml of peptone-water and 4 ml of MacConkey broth and incubating for 24 h at 44 C. The purity of the isolates was checked by parallel subculture on bile lactose agar. Isolates producing indole in peptone water and acid•plus gas in the MacConkey broth under these conditions were considered to be isolates of fecal-type *E. coli* (7). Lactose-fermenting colonies not positive in both these tests, together with any non-lactose fermenting isolates, were identified further by the method of Cowan and Steel (4).

Storage of strains. Strains for storing were subcultured onto unsupplemented bile agar plates to obtain discrete colonies. A single colony was then transferred to a nutrient agar slant, incubated overnight at 37 C, and stored at room temperature after tightening the cap.

Transfer properties of the strains. A sample (0.1 ml) of the antibiotic-resistant test strain, growing exponentially in nutrient broth, was mixed with 0.9 ml of a standard nalidixic acid-resistant recipient (*E. coli* NXR), also growing exponentially in similar medium, and the mixture was gently agitated and then incubated statically overnight at 37 C. At the end of this period the culture was centrifuged at 5,000 × *g* for 10 min, and the pellet was resuspended in 0.2 ml of nutrient broth. One loopful of the suspension was inoculated onto the surface of a bile lactose agar plate containing nalidixic acid (50 μg/ml) and the antibiotic to which the donor strain was resistant. A higher incidence of R factor transfer was sometimes achieved by repeating the test. To do this the remainder of the bacterial suspension was diluted with 10 ml of fresh nutrient broth, the culture was incubated for a further 24 h at 37 C, and the selection procedure was repeated. After overnight incubation at 37 C, individual colonies growing on the selection plates were picked, and their resistance patterns were determined.

O-antigen typing of isolated E. coli: preparation of E. coli O antigens. Only gram-negative fecal bacilli which were positive in the biochemical tests at 44 C, or which were found to be typical *E. coli* on more extensive biochemical investigation, were accepted for O-antigen typing. Discrete smooth colonies on a bile agar plate were inoculated into 4 ml of nutrient broth containing yeast extract and incubated statically overnight at 37 C. The culture was then heated for 1 h in a steamer and allowed to cool, and approximately 9 ml of crystal violet-formalin-saline solution (5 ml of formalin and 10 mg of crystal violet per liter of physiological saline) was added to give an absorbance of the resulting homogenized suspension of 0.5 at 650 nm in a Unicam SP 600 series 2 spectrophotometer.

If typing with this preparation was unsuccessful, a fresh overnight broth culture was autoclaved for 2 h at 15 lb/in², and the deposit after centrifugation (10 min, 5,000 × *g*) was suspended in 9 ml of crystal violet-formalin-saline solution. Autoclaving destroys the O-antigen-masking effect of type A capsular antigens and thus increases the number of strains typeable with O sera (29). The O-antigen masking by B- and L-type K antigens is destroyed by boiling (16,18).

Antigen preparations made in this way could be kept at room temperature.

Preparation of antisera. Antisera were made in rabbits against the standard *E. coli* strains 01 to 0153 (obtained from B. Wiedemann, Hygiene Institute, Frankfurt) by the methods of Edwards and Ewing (8). The serum was stored in 2-ml aliquots at −20 C using sodium azide as a preservative. The sera were arranged for use in 12 pools by antigenic relationships,

each pool containing specific antisera against 12 *E. coli* standard antigens. A 13th pool contained antisera against the more recently recognized *E. coli* O antigens. Stock dilutions of the sera were made in physiological saline containing 0.5% (wt/vol) phenol and stored at 4 C.

O-antigen determination. O-antigen determination is based on the method of Wiedemann and Knothe (33) adapted for use in the microtiter system.

Stage 1. O-antigen suspensions were tested against the 12 O-antisera pools in U-form Cooke microtiter plates (Cooke Engineering Co., Alexandria, Va.), 0.05 ml of antigen being added to 0.025 ml of each stock pool. The plates were then sealed and incubated for 12 to 18 h at 50 C. A negative reaction was indicated by a pellet at the bottom of the well.

Stage 2. The antigen suspension was tested against each individual serum component of the pool(s) showing a positive reaction in stage 1.

Stage 3. Although cultures belonging to certain O-antigen groups react specifically, there are many strong reciprocal and nonreciprocal antigenic relationships among cultures of different O-antigen groups. This may result in positive reactions with several pools in stage 1 and several single sera in stage 2, and it is therefore necessary to titrate sera giving positive reactions with the antigen. Doubling dilutions of sera starting at 1:50 were made, and the titer was determined after incubation overnight at 50 C.

From the pattern of reactions obtained in stages 1 and 2, and the titers in stage 3, it was possible to determine the O-antigen reaction of the original strain. For the antigen under investigation to be allocated to a particular O group with certainty it must give the same titer with specific serum (within one-well difference) as does the standard *E. coli* strain which was used to produce the serum.

If no reaction was obtained in stage 1 with antigen from boiled bacteria, the examination was repeated with an autoclaved antigen, and this antigen was also tested against pool 13. The presence of a K(A) antigen in *E. coli* strains was inferred when boiled antigen gave no reaction in any of the pools while autoclaved antigen gave positive reactions. Such a response was usually found in pools containing antisera against *E. coli* O antigens 08, 09, 0101.

If boiled antigen from a K(A)$^+$ strain of *E. coli* was tested against doubling dilutions of specific 08, 09, and 0101 sera, little or no agglutination was detected. However, after autoclaving, such a strain gave the titer of 08, 09, 0101 strains. If all the pools were negative with autoclaved bacteria, or if the reactions in stage 2 did not come up to titer with any sera in stage 3, the strain was designated nontypeable.

Some strains grown in broth agglutinate, and such preparations could not be used to produce homogenous antigen preparations. Such strains also gave positive reactions in all 12 of the stage 1 pools and also in the absence of sera. They therefore could not be O antigen typed and are termed auto-agglutinable (A).

Resistance patterns of the strains. Resistance patterns were determined by conventional techniques using a U1 multodisc (Oxoid Ltd., London) on lysed blood agar. The test plates were incubated overnight at 37 C before reading.

RESULTS

Abundance of R plasmids among the various O-antigen types of human origin. Of the 600 scored strains of *E. coli* isolated from human sources, 192 were resistant to antibiotics. They were examined for their O-antigen reactions and the results are summarized in Fig. 1b, 2b, and 3b. Initially all strains were autoclaved to remove capsular antigens, but nevertheless a substantial number of these isolates (24/192, or 12.5%) were not typeable with the 147 O-antigen sera available, and one was auto-agglutinable. Among the resistant strains that typed with O-antigen sera, the most abundant were 08 (14/192 or 7.3%), 09 (16/192 or 8.3%), and 0101 (9/192 or 4.7%). Other O-antigen types represented at above the 3% level were 020 (3.7%), 025 (3.1%), and 086 (3.1%).

Many of the strains in this series have complex resistance patterns, but this did not prove that they carry R plasmids. Accordingly, all 192 resistant isolates were tested for their ability to transfer part, at least, of their resistance pattern to a standard recipient. In all, 109/192 strains (57%) showed the ability to promote transfer in this standard test, although the complete resistance pattern of the donor was not always transferred. When transfer was correlated with the O-antigen type of the donor, the same O-antigen types were prominent as found when resistance alone was considered (Table 1). For example, 7/109 (6.4%) of the transfer-promoting strains were of serotype 08, 10/109 (9.2%) were 09, and 5/109 (4.6%) were 0101; other relatively common serotypes were 020 (4.6%), 025 (3.7%), and 086 (3.7%). The nontypeable category amounted to 13.8% of the total. Thus the overall predominance of 08, 09, and 0101 in the whole resistant collection of strains persisted when only those showing ability to transfer resistance were considered. The only additional serotype to appear above the 3% level when transferring, as opposed to merely resistant strains, was 02 (5/109 strains, or 4.6%).

Not all plasmid-carrying strains are able to promote antibiotic resistance transfer, since some carry nontransmissible plasmids (2). But to decide whether all the strains in a series as large as the one considered here carry plasmids is extremely laborious unless transfer is readily demonstrable. The presence of tetracycline resistance in a bacterial strain can, however, be taken to indicate the presence of an R plasmid since this resistance character has never been shown to be chromosomally mediated in naturally occurring strains of enteric bacteria (10, 24). Accordingly, those strains which showed tetracycline resistance in this series were added

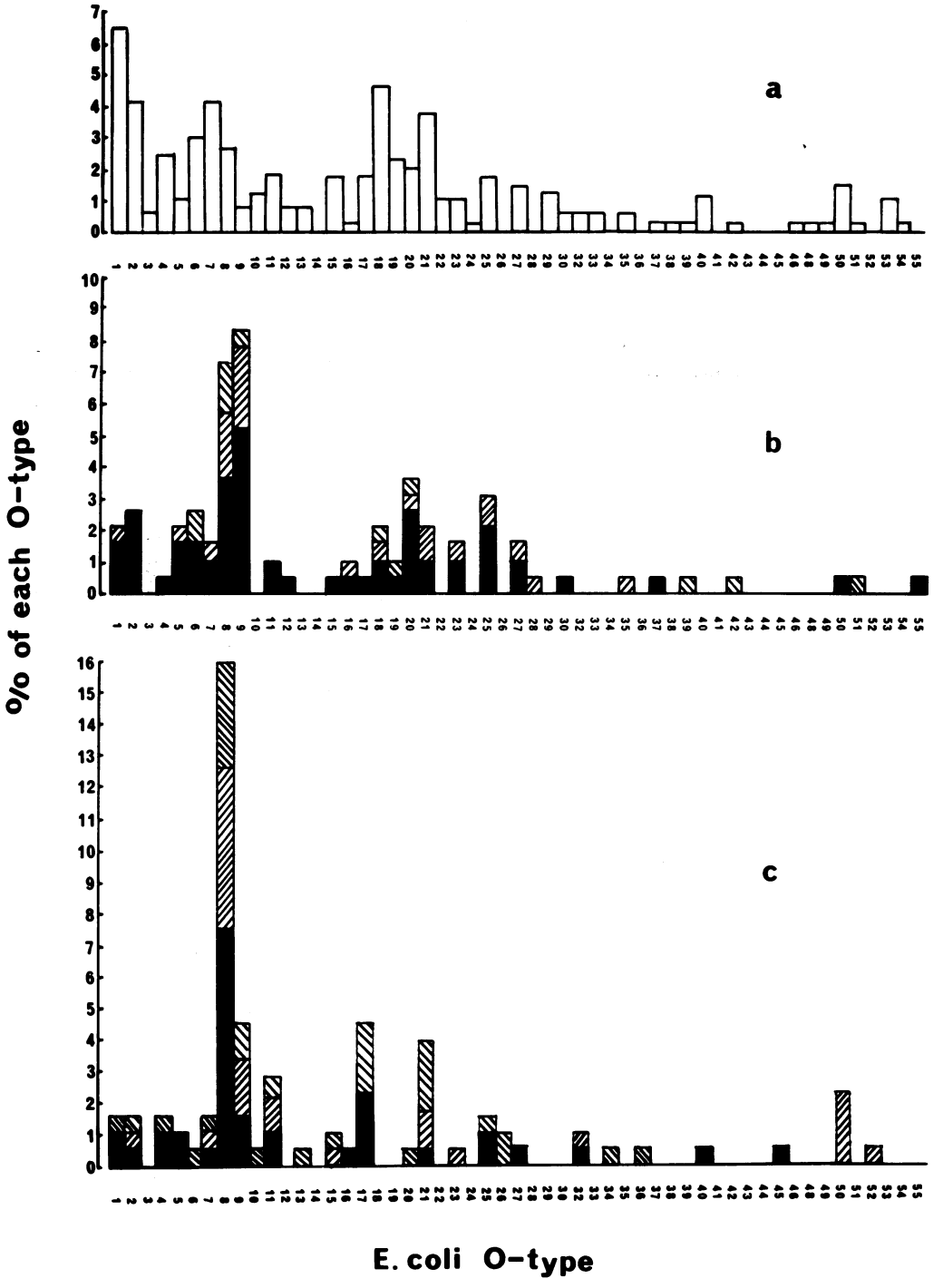

FIG. 1. *Percentage of incidence of E. coli O-antigen types (numbers 1 to 55) in feces of man and calves. Distribution a, Antibiotic-susceptible strains from man; distributions b and c, antibiotic-resistant strains from man and calves, respectively. Strains able to transfer antibiotic resistance and therefore assumed to be R plasmid-containing strains are indicated by solid black columns; tetracycline resistance, as a potential indicator of plasmid-mediated resistance, by cross-hatching from top right to bottom left, and resistance, not proven to be plasmid mediated, by cross-hatching from top left to bottom right.*

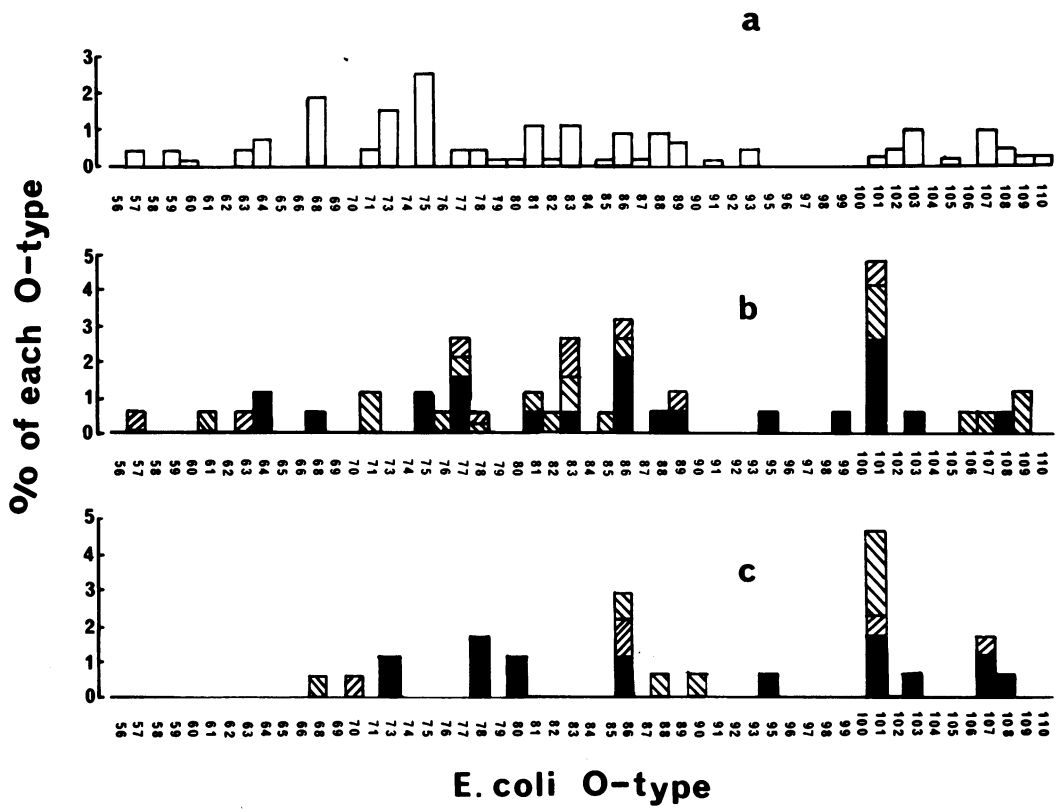

FIG. 2. *Percentage of incidence of E. coli O-antigen types in feces of man and calves (numbers 56 to 110). Key as in the legend to Fig. 1.*

to those that showed resistance transfer in the standard mating test, and this combined group of strains—which may be taken as a minimum estimate of the plasmid-carrying isolates in the series—were scored in relation to their O-antigen type. In all, 165/192 (86%) of the strains showed either direct or indirect evidence for the presence of a plasmid, and again serotypes 08, 09, and 0101 were prominent (Table 1). The first of these O-antigen types totalled 11/165 (6.7%) of the strains under discussion, and O-antigen types 09 and 0101 accounted for 9.1 and 4.8%, respectively. As with the two previous populations, the nontypeable class amounted to more than 10% of the total, and 020, 025, and 086 each accounted for about 4% of the total plasmid-carrying strains. These results show, therefore, that within this sample of independent isolates, the three O-antigen types 08, 09, 0101 together account for over 20% of the strains, regardless of whether it is plasmid carriage that is scored or merely the expression of antibiotic resistance.

O-antigen types of antibiotic-sensitive E. coli. An abundance of 08, 09, 0101 strains is not normally regarded as typical of human fecal *E. coli* isolates (9); it nevertheless seemed important to O-antigen type a range of sensitive *E. coli* isolates obtained from human fecal samples for comparison. In this case, 408 strains were O antigen typed, and the relative abundance of each O-antigen type is shown in Fig. 1a, 2a, and 3a. This distribution shows considerable differences from that found with resistant strains (cf. Fig. 1a, 2a, and 3a and 1b, 2b, and 3b). Isolates not typeable with the available O-antigen sera constituted a significant proportion of the strains (38/408, or 9.3%), and 11/408 (2.7%) were auto-agglutinable. O-antigen types 01, 02, 04, 06, 07, 08, 018, 021, and 075 all reached or exceeded the 2.5% level, with 019 and 020 close behind. Of these only 08 and 020 commonly harbored R plasmids (see Fig. 1b), and the incidence of R^+ strains among O-antigen types 01, 02, 04, 06, 07, 018, 019, 021, and 075 was substantially below what one would expect if the R plasmids were randomly distributed among *E. coli* O-antigen types.

The data summarized in Fig. 1a, 2a, and 3a, and 1b, 2b, and 3b were subjected to statistical analysis in various ways to try to determine the probability that the information contained in

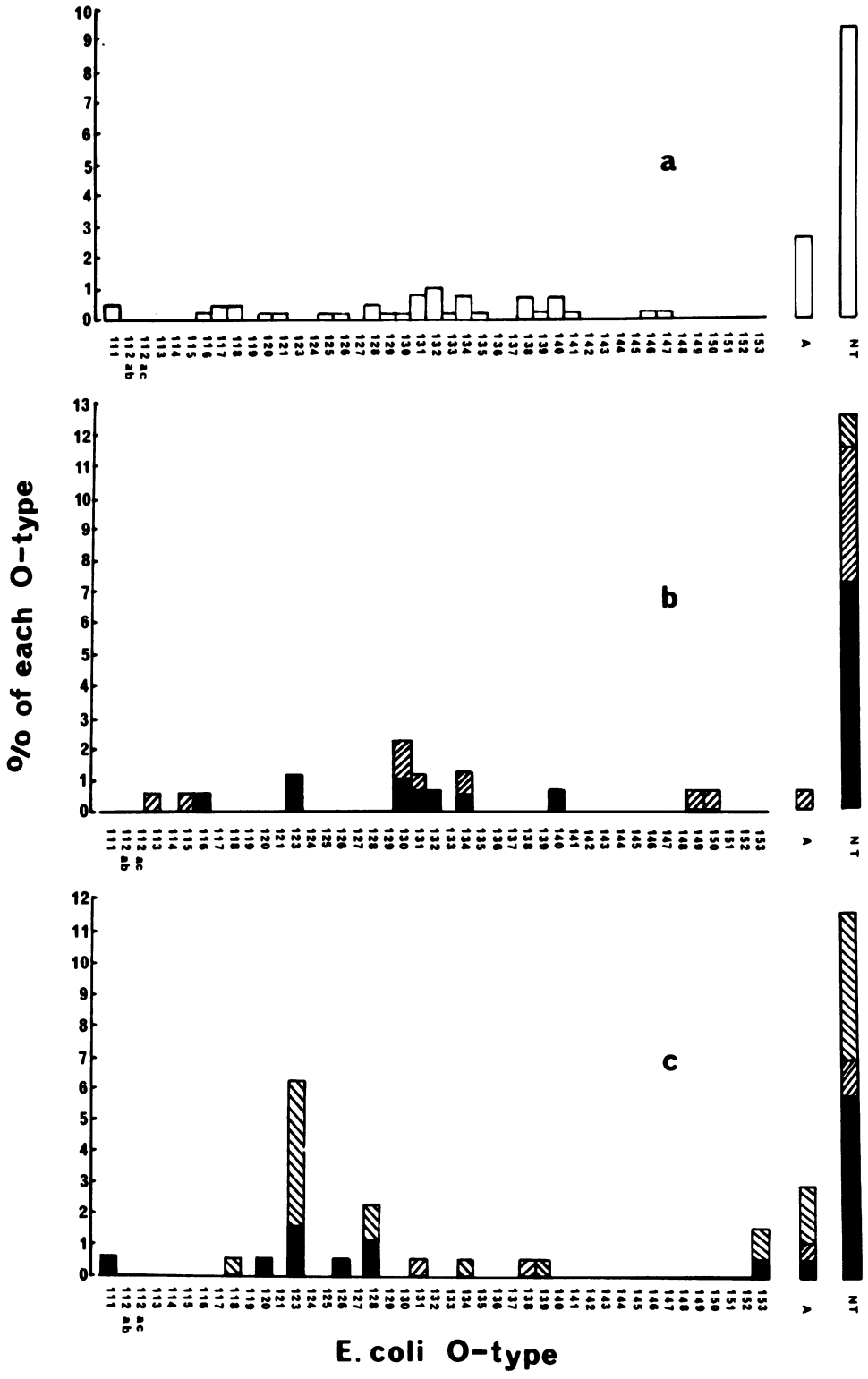

FIG. 3. *Percentage of incidence of E. coli O-antigen types (numbers 111 to 153) and of auto-agglutinable (A) and nontypable (NT) isolates from feces of man and calves. Key as in the legend to Fig. 1.*

TABLE 1. *Analysis of the various forms of antibiotic resistance within O-antigen types 08, 09, and 0101 compared with others*

E. coli O-antigen types	R plasmid demonstrated by transfer (a)	Tetracycline resistant but transfer not demonstrated (b)	Others (c)	Total (a) + (b) + (c)	%	R^+ and other tetracycline resistant-strains (a)+(b)	%
Human origin							
08	7	4	3	14	7.3	11	6.7
09	10	5	1	16	8.3	15	9.1
0101	5	3	1	9	4.7	8	4.8
Total	22	12	5	39	20.3	34	20.6
Total of all O-antigen types resistant to antibiotics	109	56	27	192		165	
Percent	56.8	29.2	14.1				
Calf origin							
08	13	9	5	27	15.5	22	20.0
09	3	3	1	7	4.0	6	5.5
0101	3	1	4	8	4.6	4	3.6
Total	19	13	10	42	24.1	32	29.1
Total of all O-antigen types resistant to antibiotics	75	35	64	174		110	
Percent	43.1	20.1	36.8				

these figures could represent different versions of the same distribution. A standard χ^2 test for this gave a P value of less than 0.01%. Similarly, a χ^2 test to measure the probability that the abundance of R^+ 08, 09, and 0101 strains could be expected from the distribution shown in Fig. 1a, 2a, and 3a gave a P value of less than 0.01%. One can have considerable confidence therefore that R plasmids are not equally abundant in all O-antigen types of *E. coli* isolated from human fecal samples.

E. coli O-antigen types 08, 09, and 0101 strains commonly carry a type A capsular antigen which must be removed by autoclaving before the O-antigen reaction can be obtained at appropriate dilution (16, 29). The 08, 09, and 0101 strains used in this study were therefore tested to decide which carried a K(A) antigen which could be removed by autoclaving. In all, 46/54 isolates in this category (36 R^+ and 10 R^-) were retested without autoclaving with O-antigen sera, and 27 (22 R^+ and 5 R^-) were found to produce much-reduced O-antigen titers. Autoclaved antigens prepared from these strains, however, gave positive reactions at the expected titers. A selection of strains showing this presumptive evidence for the presence of a capsular antigen were then tested by B. Rowe and his colleagues at the Central Public Health Laboratory, England, for their reaction with specific K sera, and all showed strong reaction with K(A) antisera (B. Rowe, unpublished data). These experiments show, therefore, that many of the 08, 09, and 0101 strains that carry R plasmids also carry a masking K(A) antigen, but they were not universally present in, nor confined to, antibiotic-resistant strains.

Pattern of isolation of the 08, 09, and 0101 strains. There is always the possibility that a group of strains emerging as do the R^+ 08, 09, and 0101 O-antigen types in this survey may be due to an unsuspected epidemic of these strains occurring during the course of the study. To check this point, the date and place of isolation of all the 08, 09, 0101 strains were investigated to see whether there was any evidence for a limited epidemic during the course of the survey. In fact these strains accumulated steadily throughout the 18 months during which collection continued and an epidemic, unless it was a very large and persistant one, seems unlikely.

Another reason for believing that this abundance of 08 and 09 strains at least does not represent a local phenomenon comes from an analysis of data of H. Knothe, W. Sietzen, and B. Wiedemann (*Proceedings of the VIIIth International Congress of Chemotherapy*, in press). Admittedly the size of their survey was relatively small. Nevertheless, within the 68 independent resistant isolates obtained in their experiments, R plasmid-carrying 08 strains occurred three times and 09 strains occurred six times, a total of 13% of the isolates. With such small numbers at issue one would not expect R^+

08 and 09 isolates to appear at all unless this class of R plasmid carrying *E. coli* was disproportionately abundant.

Do some R plasmids specify the synthesis of K(A) antigen? Even though certain of the susceptible 08, 09, and 0101 strains encountered in these surveys express the K(A) antigen, nevertheless the high proportion of resistant strains that carry this capsular antigen may imply that some R plasmids specify the synthesis of this capsular material. To test this possibility, variants of some of the resistant strains that had lost their R plasmids spontaneously were tested for the presence of the K antigen, and in all cases it was found to be still present. Similarly, transfer experiments in which R plasmids were transferred from $K(A)^+$ donors to $K(A)^-$ recipients showed no linked transfer of the resistance determinants and capsular antigens.

R plasmid carriage and lipopolysaccharide chemotype. The various O-antigen types found in *E. coli* and in *Salmonella* species fall into a number of chemotypes depending on the exact chemical composition of their lipopolysaccharide (22). It was interesting, therefore, to determine whether R plasmids were prevalent in certain of the chemotypes, or whether the chemical nature of the lipopolysaccharide layers had little to do with the carriage of resistant plasmids. Types 08 and 09 belong to the same chemotype (chemotype III), and to that extent there does appear to be a correlation. However, the other types of chemotype III (040, 058, 073, 078, and 093) do not carry R plasmids with a higher than normal frequency. At the moment, the chemical composition of the 0101 lipopolysaccharide is still unclear, but preliminary results suggest that it belongs to chemotype I (K. Jann, personal communication).

Examination of the lipopolysaccharide composition of the other O-antigen types commonly found to carry R plasmids shows that a number of chemotypes are involved. Thus 025 is of chemotype XXI, 086 is of chemotype VI, and the lipopolysaccharide structure of 020 is still unknown (21, 22). In general, therefore, there is little indication at present that there is a positive relationship between chemotype and the carriage of R plasmids.

Resistance patterns and O-antigen type. The patterns found among the resistant strains ranged from single to multiple resistance comprising five independent characters such as TASSuC (T, tetracycline; A, ampicillin; S, streptomycin; Su, sulphonamide; and C, chloramphenicol). The percentage of strains with one, two, three, four and five resistance determinants were 21, 32, 24, 13, and 10, respectively. Thus multiply resistant strains were relatively abundant within the series of isolates examined in this survey. Comparison of O-antigen type with resistance pattern revealed no significant differences, and the abundance of the various patterns within the O-antigen strains typing as 08, 09, and 0101 was not significantly different from the relative abundance of the same pattern among the remaining O-antigen types and the nontypeable strains.

Abundance of various O-antigen types among the resistant strains of animal origin. In view of the possible public health importance of resistant *E. coli* strains in farm animals (15, 26, 28), it was of particular interest to establish whether the O-antigen types commonly associated with R plasmid carriage in the human fecal flora also occurred in farm animals. A number of *E. coli* strains isolated from calves being reared by standard methods of husbandry were therefore examined. Of 264 scored isolates from calf feces, 174 were resistant to antibiotics and were typed with specific O-antigen sera (see Fig. 1c, 2c, and 3c). As with the human strains, all isolates which could not be typed after boiling were autoclaved to remove the capsular antigens, but this still left 20/174 strains (11.5%) which were not typeable with the O-antigen sera available. This percentage is close to the proportion of human strains (12.5%) that fell in this same category. This argues that resistant *E. coli* from man and from calves are about equally typeable with the 147 O-antigen sera available for this work. The most abundant resistant strains in this study were 08, 09, 011, 017, 021, 050, 086, 0101, 0123, and 0128. Of these, 08, 09, and 0101 together constituted 42/174 strains (24.1%) (Table 1). Of the O-antigen types found relatively commonly among antibiotic-susceptible and -resistant human fecal *E. coli*, 086 (1.7%) in man was also common in the animal material (2.7%). On the other hand, resistant strains of O-antigen types 0123 and 0128, which were strikingly abundant in calves (6.3% and 2.8%, respectively), were uncommon among human strains encountered in this survey (cf. Fig. 3b and 3c).

A comparison of the typing of 08, 09, and 0101 strains of animal origin before and after autoclaving showed that 55.9% of the strains from calves carried capsular type A antigen. This value was similar to that obtained with the strains of these O-antigen types from human feces (58.7%).

DISCUSSION

The differences between the relative abundance of the susceptible and R plasmid-carrying

O-antigen types in man raises many interesting questions. The chance of finding an R plasmid-carrying strain of given O-antigen type in a fecal sample from a person not receiving antibiotics presumably reflects a complex situation dependent on many factors. Among these are the abundance of the particular strain in the food, its ability to multiply in various parts of the gastrointestinal tract, and its ability to resist elimination. The prevalence of resistance will reflect the facility with which the strain acquires R plasmids, the ease with which it loses them, and the influence that the R plasmid itself may have on the colonizing properties of the strain. It is already known from laboratory experiments that *E. coli* strains differ in many of these respects (17), and that plasmids may further influence some of these properties (36). In view of the relative prevalence of R plasmid carrying 08, 09, and 0101 strains in man and calves, a detailed study of the survival characteristics of this group of *E. coli* O-antigen types in man and animals now seems worthwhile.

Another feature that is difficult to understand clearly at the moment is why *E. coli* O-antigen types 08, 09, and 0101 should so frequently carry R plasmids when compared with other *E. coli* O-antigen types. These three O-antigen types are known to be related by the fact that they are the only types ever to be associated with the carriage of A-capsular antigens (22, 24). However, not all strains of these O-antigen types, resistant or susceptible, actually carry such a capsular antigen, and there does not seem, therefore, to be a simple correlation between the presence of a K(A) antigen and R plasmid carriage.

The distributions in Fig. 1a, 2a, and 3a and 1b, 2b, and 3b show that many of the O-antigen types regarded as good colonizers of the human gut—notably 01, 02, 021, 025, and 075—carry R plasmids less commonly than would be expected on a random basis. This agrees with the findings of Kauffmann (17). This presumably accounts for the fact that although R plasmid carriage is common among fecal *E. coli* in man, the R plasmid-carrying strains rarely become part of the dominant flora unless selection pressure is applied. Were R plasmid carriage to become a more common feature of some of the colonizing *E. coli* O-antigen types one might anticipate an increase in R plasmid carriage among *E. coli* strains in human fecal samples, and, moreover, this increase might have little to do with antibiotic use. This stresses an important point in R plasmid ecology: antibiotic use is not the only selective influence that operates on these elements. The colonizing properties of the host strains also play a major part.

There has been much discussion about the extent to which antibiotic-resistant *E. coli* in farm animals pose a threat to human health (15, 26, 28), and there have been attempts to question their importance on the grounds that the human and animal populations are distinct. The data collected in the surveys reported here suggest, on the contrary, that man and calves (at least) share many *E. coli* O-antigen types among their antibiotic-resistant strains and that it is consequently unwise to rule out organisms of animal origin as a source of human fecal strains. Gruneberg et al. have shown—without being primarily concerned with the resistance patterns of the bacteria (see reference 11)—that the serological typing patterns of *E. coli* of human fecal strains are indistinguishable, in many cases, from those organisms isolated from human urinary-tract infections, and Davies et al. have shown that resistant strains infecting burns commonly originate in the gut of the person concerned (6). Moreover, V. Petrocheilou (in unpublished experiments from this department) has shown that R plasmid-carrying 08, 09, and 0101 strains of *E. coli* may be isolated from urinary-tract infections in humans. Therefore, although the evidence presented so far does not prove that *E. coli* strains causing disease in man originate in farm animals, the possibility certainly cannot be excluded on the grounds of the difference of the types concerned.

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